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Competitive Exclusion of *Salmonella* from the Gut of Neonatal and Weaned Pigs

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ABSTRACT

Our laboratory has developed a bacterial competitive-exclusion (CE) culture against enteropathogens (which are considered human foodborne pathogens) for use in swine. In this article, we document the effects of this CE culture, PCF1, on cecal colonization by and fecal shedding of *Salmonella* Choleraesuis in neonatal and weaned pigs and its effects on the horizontal transmission of this pathogen between weaned penmates. Piglets treated with the PCF1 culture twice within their first day of life and challenged with *Salmonella* 48 h after birth shed *Salmonella* at a significantly ($P < 0.05$) lower rate than did control pigs in experiment 1. Significant reductions of the pathogen were also observed in the cecum, the cecal contents, the ileocolic junction, and the colon contents ($P < 0.05$). In experiment 2, culture of the cecal contents and lymph nodes revealed a significant reduction in *Salmonella* isolated from PCF1-treated pigs ($P < 0.05$). Pigs in experiment 3 were treated as pigs in experiments 1 and 2 were; however, they were followed through day 10 postweaning. Significant reductions in shedding were noted for treated groups both pre- and postweaning ($P < 0.05$). Experiments 4 and 5 assessed the effects of PCF1 treatment on the horizontal transmission of *Salmonella* between littermates that were followed through day 14 postweaning. In these experiments, litters were divided into untreated contacts (UC), untreated seeders (US), treated contacts (TC), and treated seeders (TS). Overall, TC in experiment 4 shed *Salmonella* at a significantly lower rate than UC and US did ($P < 0.05$). In experiment 5, the transmission of *Salmonella* was significantly reduced for litters in which TS or TC were present, as evidenced by reduced shedding of *Salmonella* by both treated and untreated animals within these litters ($P < 0.05$). TS shed less often than US did, resulting in reduced levels of *Salmonella* shedding by both treated and untreated contacts ($P < 0.05$). Litters containing both TC and UC or both TC and US also shed *Salmonella* at lower rates than did litters in which only UC and US were present ($P < 0.05$).

Salmonella has been isolated from nearly all vertebrate hosts from which it has been sought, with the possible exception of healthy fish in unpolluted water. Swine, cattle, and poultry are known carriers of salmonella *(7, 31, 41)*. *Salmonella* has also been associated with foodborne illness in humans *(7)*. Humans are typically infected with salmonellae through the ingestion of contaminated food or food products, and infection with salmonellae usually results in severe gastroenteritis *(22)*. Transmission of the pathogen among swine can occur through the fecal-oral and intranasal routes, involving colonization of and dissemination from the gastrointestinal tract and organs such as lungs and tonsils, respectively *(18)*.

Salmonella colonizes and inhabits the ceca of swine *(14)*, and in poultry the cecum has been shown to be the primary site of salmonella colonization *(28)*. The cecal environments for adult swine and poultry are similar in terms of pH, oxidation reduction potential, anaerobicity, and bacterial populations because swine and poultry are both hindgut fermenters and are fed very similar diets under commercial production conditions. The digestive tract of the newborn pig is usually sterile but rapidly develops a microflora characteristic of the species as the pig is exposed to a traditional commercial environment *(23)*. Essentially sterile at the time of hatching, the intestinal tracts of poultry are rapidly colonized by microorganisms from the environment *(29)*. The presence of a stable gastrointestinal microflora aids an animal in resisting infections, particularly in the gastrointestinal tract *(26)*. This phenomenon has been referred to as bacterial antagonism *(19)*, bacterial interference *(16)*, the barrier effect *(17)*, colonization resistance *(49)*, and competitive exclusion (CE) *(32)*.

The mechanism by which indigenous gut flora prevent salmonella colonization is not clear. Lloyd et al. *(32)* proposed that normal gut flora adhere to the intestinal-cecal epithelial cells and exclude salmonellae from essential microhabitats. Snoeyenbos et al. *(43, 44)* suggested that protection is due to direct competition for attachment sites. Soerjadi et al. *(45, 46)* reported that native gut flora adhere to the cecum as a mat of interconnected cells that may prevent the attachment of salmonellae, while Snoeyenbos et al. *(44)* suggested that in addition to adherence competition, normal gut flora metabolites may contribute to salmonella colonization control. Nisbet et al. *(36)* demonstrated that the oral administration of a CE culture rapidly established itself in the ceca of newly hatched chicks and

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resulted in a 100-fold increase in microbial populations for 3-day-old chicks compared with those for untreated controls. Additionally, this increase was highly correlated with increased concentrations of cecal total volatile fatty acids, especially propionic acid, and reductions in *Salmonella* Typhimurium cecal colonization levels. Furthermore, with the use of electron scanning microscopy it was shown that the CE bacteria preferentially colonized the crypts of the cecal mucosal epithelium, a primary site of salmonella colonization and invasion *(15).* The production of short-chain volatile fatty acids by anaerobic bacteria in the ceca was reported to inhibit *Salmonella* colonization in mice *(9)* and has been proposed to inhibit enteropathogens in poultry *(5, 6)* and swine *(39, 40).* The gastrointestinal volatile fatty acid profiles for pigs at weaning have been shown to decrease during the first 10 days postweaning, a period associated with enteropathogen colonization *(33).* The bacterial microflora in the mouse has been shown to undergo distinct changes as a result of weaning, and enteropathogen control in the gastrointestinal tracts of weaned mice has been associated with the concentration of butyric acid *(32).* Undissociated volatile fatty acids have also been reported to have an anti-enteropathogen effect in swine *(40).* Competition between normal flora and *Salmonella* for limited nutrients has also been proposed to be a mechanism that may control *Salmonella* growth *(4–6, 26).* In studies involving the use of continuous-flow (CF) cultures as models of the mouse intestinal ecosystem, Freter et al. *(20, 21)* and Wilson and Freter *(50)* proposed that the population dynamics of normal flora and invading enteropathogens may be regulated by competition for one or a few limiting nutrients.

Recently, with the use of microflora obtained from adult swine, our laboratory has employed a CF culture technique previously approved for use in the commercial poultry industry to develop a CE culture for swine *(35, 37).* Anderson et al. *(3)* have demonstrated that treating pigs with this CF culture of mixed species of bacteria present swine microflora (PCF1) decreases the concentration and incidence of *Salmonella* Choleraesuis in the ceca of weaned pigs and decreases fecal shedding. In this report, we document the effects of PCF1 on cecal colonization by and fecal shedding of *Salmonella* Choleraesuis in baby and weaned pigs and on the horizontal transmission of this pathogen among weaned penmates.

MATERIALS AND METHODS

CF culture. The porcine-derived CF culture (PCF1) was propagated from cecal contents collected from a 6-week-old healthy pig and was maintained through CF culture as described for avian cultures *(12, 13).* Briefly, cecal contents (ca. 50 g) were collected from the pig (which had been obtained from a commercial producer and maintained in our facility on a typical commercial diet that was free of antibiotics) and immediately transferred to an anaerobic chamber (Coy Laboratory Products, Ann Arbor, Mich.). The cecal contents were added to 100 ml of anaerobic Viande Levure broth medium (tryptose [10 g/liter], beef extract [2.4 g/liter], yeast extract [5 g/liter], dextrose [2.5 g/liter], and NaCl [2.5 g/liter]), and this mixture was immediately used as a seed culture for the CF culture system. For CF culture of the mixed cecal microflora, a BioFlo I fermenter fitted with a 2,000-ml chemostat vessel with an 1,150-ml working volume was used (New Brunswick Scientific Co., Edison, N.J.). The chemostat vessel containing 1,000 ml of Viande Levure broth was constantly flushed with a stream of O_2-free CO_2 to maintain anaerobic conditions. The medium was prepared in 13-liter Pyrex bottles, autoclaved for 1.5 h, and flushed with a constant stream of O_2-free CO_2 immediately upon removal from the autoclave. The chemostat vessel was filled with 1,000 ml of the Viande Levure medium (pH 5.5) and allowed to sit for 48 h before inoculation to ensure that there had been no microbial contamination prior to inoculation. The vessel was inoculated with the above-mentioned inoculum, the nutrient pump was turned on, and the culture was incubated under CF conditions. The dilution rate for the CF culture was 0.0416 per h. The CF culture was monitored daily for fermentation products and pH. After five vessel turnovers, a constant pH (6.0 to 6.2) was observed and the culture was deemed to be in a steady-state condition. This method of maintaining CF cultures has previously been used in our laboratory for the successful maintenance of CF cultures of poultry gut origin *(42, 43).* The PCF1 culture *(38)* contains at least seven of the following bacterial species: *Enterococcus faecalis, Streptococcus bovis, Clostridium clostridiforme, C. symbiosurn, C. ramosum, Bacteroides fragilis, B. distasonis, B. vulgatus, B. uniformis,* and *B. caccae*. However, the culture is not limited to these species.

Salmonella. *Salmonella* used for experimental challenges was propagated from a primary pig isolate of *Salmonella* Choleraesuis var. Kunzendorf $_x$3246. This isolate was selected on the basis of its resistance to both novobiocin (NO) and nalidixic acid (NA) in our laboratory and was maintained in tryptic soy broth medium containing NO at 25 μg/ml and NA at 20 μg/ml. All experimental *Salmonella* challenge materials were prepared from an overnight culture that had been serially cultured two consecutive times at 37°C for 24 h each. The challenge doses of *Salmonella* Choleraesuis were determined on the basis of viable cell counts following overnight incubation at 37°C on brilliant green agar (BGA; Oxoid, Unipath Ltd., Basingstoke, Hampshire, UK) supplemented with NO at 25 μg/ml and NA at 20 μg/ml ($BGA_{NO/NA}$).

Animal experiments 1 and 2. Pregnant sows were purchased from a commercial producer and maintained in our laboratories on a commercial diet that was free of antibiotics. Sows were cultured for the presence of wild-type salmonellae on arrival and up to the time of experimental challenge by previously described methods *(30).* With the exception of swabs from sows and baby pigs in experiment 1 (see "Results"), wild-type salmonellae were not detected in rectal swabs collected from sows prior to farrowing or from piglets for the 2 days immediately preceding experimental challenge. The prechallenge swabs were cultured via preenrichment in GN-Hajna broth (Difco Laboratories, Sparks, Md.), further enrichment in Rappaport-Vassiliadis broth (Difco), and selective differentiation on BGA plates containing NO (BGA_{NO} plates) *(27).* Treated pigs were provided with a 5.0-ml oral dose of the PCF1 culture (10^9 CFU/ml of culture) from a sample that was withdrawn from the fermenter and transferred into sterile O_2-free serum bottles. The culture was given to treated pigs within 30 min of withdrawal from the fermenter. Treatment was provided within 4 h of birth and again 24 h later. Piglets were challenged by intranasal inoculation with 2 ml of NO- and NA-resistant *Salmonella* Choleraesuis (10^3 CFU/ml) 48 h after PCF1 treatment in experiment 1. Control piglets were challenged similarly; however, no PCF1 treatment was provided. One week postchallenge, piglets were euthanatized by injection with sodium pentobarbital and necropsied for the collection of ileocolic lymph nodes, cecal contents, and, in some of the experiments, tonsils,

ileocolic junctions, livers, spleens, lungs, and colons. Rectal swabs, tissues, and cecal contents collected from piglets after challenge were incubated overnight at 37°C in GN-Hajna broth, transferred to Rappaport-Vassiliadis broth and incubated overnight at 37°C, and then streaked on $BGA_{NO/NA}$ to culture for *Salmonella* Choleraesuis. Plates were examined for colonies exhibiting typical salmonella morphological characteristics, and suspect colonies were confirmed via serum agglutination with the use of *Salmonella* Antiserum Poly A I-IV and Group C_1, Factors 5 and 6 (Difco). Several representative colonies were also sent to the National Veterinary Services Laboratory (Ames, Iowa) for serotyping, and all colonies were confirmed to be *Salmonella* Choleraesuis var. Kunzendorf. In experiment 2, piglets were handled in a similar manner but were challenged via oral administration of 2 ml of NO- and NA-resistant *Salmonella* Choleraesuis (10^3 CFU/ml). Cecal contents were serially diluted in phosphate-buffered saline and were then spread plated on $BGA_{NO/NA}$ plates, and *Salmonella* counts were obtained. All animals were cared for according to standard swine husbandry practices and were fed (ad libitum) a typical commercial diet (corn and soy bases) formulated to be free of antibiotics and to meet or exceed nutrient requirements.

Animal experiments 3 and 4. Sows and piglets in experiments 3 and 4 were subjected to the same animal husbandry practices used in experiments 1 and 2. Sows and piglets were determined to be free of wild-type salmonellae prior to the experimental challenge. Treated piglets were provided the PCF1 culture within 4 h of farrowing and again 24 h later. In experiment 3, all piglets were orally challenged with 10^5 CFU of *Salmonella* Choleraesuis; on day 14, piglets were weaned from the sows and litters were housed in separate pens. Rectal swabs were taken daily from the day after experimental challenge until the termination of the experiment (10 days postweaning), and *Salmonella* incidences and cecal concentrations were determined as in experiments 1 and 2. In experiment 4, treated pigs were provided only one dose of the PCF1 culture, and this dose was provided within 4 h of birth. Experiment 4 was designed to measure the effects of horizontal transmission on the incidence of *Salmonella* shedding, which was determined with the use of daily rectal swabs. In the first litter of pigs, the piglets were divided into two groups, designated contacts and seeders. Seeder piglets were orally challenged with 10^7 CFU of *Salmonella* Choleraesuis 48 h after birth, and the remaining piglets were designated contact piglets and were unchallenged; neither group in litter 1 was provided PCF1. This experiment was carried out to determine the extent of horizontal transmission of the pathogen from infected to noninfected pigs. Rectal swabs were taken from piglets daily from the day they were born until the end of the experiment. Piglets were weaned at 14 days of age, and the litter was housed in a pen. Litters 2 and 3 were handled in the same manner except that these litters were divided into three different groups; seeders, untreated contacts, and PCF1-treated contacts. For these litters, PCF1 was provided to the treated contact piglets once within 4 h of birth, and the seeder piglets were challenged with *Salmonella* Choleraesuis 24 h later. This experimental design allowed us to determine the effect of PCF1 on the horizontal transmission of *Salmonella* Choleraesuis from the challenged seeder pigs to the untreated and treated contacts and was chosen because it best represents what most likely occurs under commercial conditions in the swine industry. For all three litters in experiment 4, ear tags were used to identify the individual piglets as seeders, untreated contacts, or treated contacts.

Animal experiment 5. Sows and piglets were subjected to the husbandry practices described above. Experiment 5 was conducted to assess the effects of a single dose of PCF1 within 4 h of the birth of pigs on the horizontal transmission of *Salmonella* Choleraesuis between littermates. Litters were as follows: litter 1 ($n = 6$)—seeders, untreated contacts; litter 2 ($n = 8$)—seeders, PCF1 contacts; litter 3 ($n = 8$)—PCF1 seeders, PCF1 contacts; litter 4 ($n = 5$)—PCF1 seeders, untreated contacts; litter 5 ($n = 13$)—seeders, untreated contacts, PCF-1 contacts; litter 6 ($n = 10$)—PCF1 seeders, untreated contacts, PCF1 contacts. Both untreated seeders and PCF1 seeders were orally administered 2 ml of *Salmonella* Choleraesuis (10^7 CFU/ml) 48 h after birth. Beginning the day after *Salmonella* challenge, rectal swabs were taken daily from each pig and examined for the presence of *Salmonella* Choleraesuis. Seeders, untreated contacts, treated seeders, and treated contacts were divided within the litters. Piglets were then handled as described in experiment 4.

Data analysis. Data were analyzed with Sigma Stat software (Jandel Scientific, San Rafael, Calif.). Significant differences in the numbers of *Salmonella* CFU between groups were determined by the *t* test. Chi-square analysis was performed to determine differences in the numbers of *Salmonella*-positive samples for different groups.

RESULTS

Experiment 1. Analysis of rectal swabs indicated that sows were free of salmonellae when they first arrived at the facility. However, prior to farrowing, the control sow was shedding a serogroup B *Salmonella.* In addition, the piglets of both the control sow and the treated sow were also determined to be shedding a wild-type serogroup B *Salmonella.* No further identification of the serogroup B *Salmonella* was carried out. Piglets were challenged with the *Salmonella* Choleraesuis (serogroup C_1) challenge organism (with the use of intranasal administration); therefore, the *Salmonella* incidence data obtained in experiment 1 represent total salmonellae and not just the *Salmonella* Choleraesuis challenge organism. The control piglets shed the serogroup B *Salmonella* exclusively, as determined by the analysis of rectal swabs, whereas in tissue samples there was a mixture of the challenge organism (*Salmonella* Choleraesuis) and the serogroup B *Salmonella.* For the PCF1-treated piglets, a mixture of the wild-type serogroup B *Salmonella* and the challenge *Salmonella* (*Salmonella* Choleraesuis) was found in the rectal swabs, but only *Salmonella* Choleraesuis was isolated from tissue samples. No consistent differences between the incidence of *Salmonella*-positive tissue samples for treated piglets and that for control piglets with respect to treatment were observed (data not shown). For the gut samples, total *Salmonella* incidences were significantly reduced ($P < 0.05$) in the ceca, in the cecal and colonic contents, and in the ileocolic junctions of treated piglets compared with those for control piglets (Table 1).

Experiment 2. Sows and piglets used in experiment 2 were determined to be free of wild-type salmonellae prior to experimental challenge. The *Salmonella* Choleraesuis challenges in this experiment and in the remaining experiments were administered orally, whereas in experiment 1 the challenge was administered via intranasal instillation. Treated piglets did not shed *Salmonella* Choleraesuis at any time during the experiment, as indicated by rectal swab

TABLE 1. *Effects of porcine competitive-exclusion culture on incidence of gut colonization by, and fecal shedding of,* Salmonella *in suckling pigs*[a]

Sample	No. of pigs positive for *Salmonella*/ no. of pigs tested (%)	
	Control pigs	Treated pigs
Rectal swab	72/72 (100) A	10/56 (18) B
Cecum	9/9 (100) A	0/7 (0) B
Cecal contents	9/9 (100) A	2/7 (29) B
Ileocolic junction	9/9 (100) A	1/7 (14) B
Colonic contents	6/9 (67) A	0/7 (0) B

[a] Treated pigs were provided an oral dose of 5.0 ml of the porcine competitive-exclusion (PCF1) culture containing 10^9 CFU/ml within 4 h of birth and again 24 h later. All treated pigs were intranasally challenged 48 h after the second PCF1 dose with 10^3 CFU of *Salmonella* Cholerasuis. Control pigs were similarly challenged 72 h after birth. Values with different letters in the same row are significantly different ($P < 0.05$).

TABLE 2. *Effects of porcine competitive-exclusion culture on incidence of gut colonization by* Salmonella *Cholerasuis in suckling pigs*[a]

Sample	No. of pigs positive for *Salmonella* Cholerasuis/ no. of pigs tested (%)	
	Control pigs	Treated pigs
Rectal swab (incidence of shedding)	4/90 (4.4) A	0/72 (0) A
Rectal swab (pigs shedding *Salmonella* Cholerasuis at least once)	3/10 (30) A	0/1 (0) B
Ileocolic lymph nodes	10/10 (100) A	3/8 (38) B
Cecal contents	8/10 (80) A	3/8 (38) B

[a] Treated pigs were provided an oral dose of 5.0 ml of the porcine competitive-exclusion (PCF1) culture containing 10^9 CFU/ml within 4 h of birth and again 24 h later. All treated pigs were intranasally challenged 48 h after the second PCF1 dose with 10^3 CFU of *Salmonella* Cholerasuis. Control pigs were similarly challenged 72 h after birth. Values with different letters in the same row are significantly different ($P < 0.05$). *Salmonella* Cholerasuis counts for the cecal contents of control and treated pigs were significantly different at 2.9 ± 1.97 $\log_{10}$ CFU/g (range, 0 to 6.7 $\log_{10}$ CFU/g) and 1.2 ± 1.7 $\log_{10}$ CFU/g (range, 0 to 3.8 $\log_{10}$ CFU/g), respectively ($P < 0.05$).

data. However, incidences of the shedding of total *Salmonella* were not significantly different ($P > 0.05$) between control piglets and treated piglets, because a low incidence of shedding was observed for the control piglet group (Table 2). Incidences of *Salmonella* Choleraesuis in ileocolic lymph nodes and cecal contents were significantly lower ($P < 0.05$) for treated piglets than for control piglets. In addition, *Salmonella* Choleraesuis counts for cecal contents were >90% lower for treated piglets than for control piglets ($P > 0.05$).

Experiment 3. Wild-type salmonellae were not isolated from either sows or piglets in experiment 3. Piglets in group 1 shed *Salmonella* at a significantly lower rate than did the challenge control pigs during the preweaning phase ($P < 0.05$) (Table 3). There was no difference between the shedding rate for control pigs and that for treated pigs in group 2 during the preweaning phase of the experiment. After weaning, both group 1 and group 2 pigs shed *Salmonella* at significantly lower rates ($P < 0.05$) than the controls did. The *Salmonella* count for the cecal contents of group 2 was >2.5 $\log_{10}$ units lower than that for those of control pigs, with none of the group 2 pigs culturing positive for *Salmonella* in either the cecal contents or the cecum itself. There was no difference in *Salmonella* counts for cecal contents for group 1 compared to controls.

Experiment 4. Wild-type *Salmonella* was not isolated from pigs during experiment 4. The numbers of positive rectal swab samples for treated contacts were significantly smaller than those for untreated contacts and seeders overall ($P < 0.05$) (Table 4). Numbers of positive samples for treated contacts were significantly smaller than those for seeders throughout the study ($P < 0.05$) (Table 4). However, although overall reductions in numbers of positive rectal swab samples for treated contacts were observed when all three litters in experiment 4 were compared, significant differences between the numbers for treated contacts and untreated contacts within litters 2 and 3 were not observed (Table 4). However, differences between total percentages of untreated and treated contacts were significant ($P < 0.05$).

Experiment 5. The results of experiment 5 are presented in Table 5. Sows and piglets were found to be free of wild-type *Salmonella.* Rates of *Salmonella* shedding for treated contacts were significantly lower than those for both treated seeders and untreated seeders, as indicated by analysis of daily rectal swabs ($P < 0.05$). Overall, treated seeders shed *Salmonella* significantly less often than did untreated seeders ($P < 0.05$). Treated contacts also shed *Salmonella* significantly less often than did untreated contacts when both treated and untreated contacts were present in a litter and when either group was present individually in a litter ($P < 0.05$).

DISCUSSION

The results of the present studies suggest that the use of a mixed-bacterial-species CE culture can reduce both the *Salmonella* count in the gut and the fecal shedding of these pathogens into the environment. Reductions in the fecal shedding of *Salmonella* resulted in diminished horizontal transmission between pen- and littermates. The reductions in *Salmonella* counts observed for PCF1-treated pigs and for pigs that came into contact with them may translate to less contamination in the slaughter plant and subsequent reductions in the contamination of pork products destined for human consumption.

Previous experiments conducted in our laboratory and elsewhere have demonstrated the efficacy of CE cultures (mixed- and single-strain cultures) in protecting swine against the enteropathogens *Salmonella* and *Escherichia coli (3, 17, 24, 25, 47, 48)* (see Blanco et al. *(8)* for *E. coli* virulence factors). Studies using single strains of *Streptococcus* spp. as microbial prophylactics against *E. coli* in

TABLE 3. *Effects of porcine competitive-exclusion culture on fecal shedding of* Salmonella *Cholerasuis in pigs pre- and postweaning and on* Salmonella *cecal colonization in weaned pigs*[a]

Sample	No. of pigs positive/no. of pigs tested (%)		
	Controls ($n = 6$)	Group 1 ($n = 8$)	Group 2 ($n = 4$)
Rectal swab (fecal shedding preweaning)	26/57 (46) A	8/63 (13) B	12/28 (43) A
Rectal swab (fecal shedding postweaning)	61/102 (60) A	74/148 (50) B	17/56 (30) C
Cecal contents (incidence of *Salmonella* Cholerasuis–positive pigs)	4/6 (67) A	5/8 (63) A	0/4 (0) B

[a] Treated pigs (groups 1 and 2) were provided an oral dose of 5.0 ml of the porcine competitive-exclusion (PCF1) culture containing 10^9 CFU/ml within 4 h of birth and again 24 h later. All treated pigs were intranasally challenged 48 h after the second PCF1 dose with 10^5 CFU of *Salmonella* Cholerasuis. Control pigs were similarly challenged 72 h after birth. Values with different letters in the same row are significantly different ($P < 0.05$). *Salmonella* Cholerasuis counts for the cecal contents of controls (2.81 ± 2.34 $\log_{10}$ CFU/g; range, 0 to 5.8 $\log_{10}$ CFU/g) and for the cecal contents of group 1 (1.65 ± 1.13 $\log_{10}$ CFU/g; range, 0 to 3.5 $\log_{10}$ CFU/g) were significantly different ($P < 0.05$) from the count for group 2 (0 ± 0 $\log_{10}$ CFU/g).

swine were conducted in either gnotobiotic or caesarian-derived, colostrum-deprived pigs *(47, 48).* Experiments in our laboratory have involved the use of pigs farrowed and raised with the use of traditional swine production practices. The incorporation of traditional rearing practices into these studies allows the simulation of most, but not all, of the characteristics of a commercial swine system, and hence the results of these studies represent results that might be obtained if the studies were conducted on a commercial hog farm.

As in our work with CE in chickens, reduced horizontal transmission was observed for swine *(11, 37).* Anderson et al. *(1, 2)* found that the incidence of the transmission of *Salmonella* Choleraesuis by experimentally infected pigs was high (4 of 10 pigs exposed) when an oral dose of 10^8 CFU was given to seeder pigs, but no transmission was observed for seeder pigs given lower doses. The present studies indicate that the administration of a dose of 10^7 CFU of *Salmonella* Choleraesuis to seeder pigs was sufficient to result in transmission from seeder pigs to contacts. The rates of transmission of *Salmonella* were low for untreated contacts and greatly reduced for treated contacts (Tables 4 and 5). The apparent low incidence of the transmission of *Salmonella* Choleraesuis between swine in laboratory studies involving low doses of the bacteria makes seeder-contact studies difficult and results in the use of much higher doses of bacteria than are likely to be encountered in the production environment in order to enable researchers to measure differences in the rates of transmission between treated and untreated animals. Significant ($P < 0.05$) reductions in *Salmonella* counts for lymph nodes and cecal contents (Tables 1 and 2) were observed for treated pigs in experiments 1 and 2. In studies involving similar conditions and a challenge dose 10^3 CFU of *Salmonella* Choleraesuis, Fedorka-Cray et al. *(17)* found similar reductions in the gut after piglets were administered a mucosal CE culture (MCES) soon after birth. Reductions in numbers of enteropathogens during the pre- and postweaning periods may translate to reductions in the overall contamination of pork products in the processing plant, although this possibility has not been investigated. Poultry studies suggest that reductions in *Salmonella* counts for young chickens translate to lower *Salmonella* counts for chickens prior to slaughter *(10).* Further studies will be conducted to investigate whether reductions in enteropathogens are seen at the finishing stage of

TABLE 4. *Effects of porcine competitive-exclusion culture on horizontal transmission of* Salmonella *Cholerasuis between littermates*[a]

Group	No. of rectal samples of *Salmonella* Cholerasuis positive/no. of rectal samples tested (%)			
	Litter 1 ($n = 8$)	Litter 2 ($n = 6$)	Litter 3 ($n = 9$)	Total
Seeders	46/71 (65) A	37/46 (80) A	29/63 (47) A	112/180 (62) A
Untreated contacts	33/88 (38) B	3/46 (7) B	7/63 (11) B	43/197 (21) B
Treated contacts	NP[b]	2/46 (4) B	8/63 (13) B	10/109 (9) C

[a] Treated contact pigs were provided an oral dose of 5.0 ml of the porcine competitive-exclusion (PCF1) culture containing 10^9 CFU/ml within 4 h of birth. Untreated contact pigs were neither challenged with *Salmonella* Cholerasuis nor provided PCF1. Seeder pigs were challenged with 10^7 CFU of *Salmonella* Cholerasuis. Piglets remained on sows until day 14 and were then weaned; individual litters were housed in separate pens until the termination of the experiment. Values with different letters in the same column are significantly different ($P < 0.05$).

[b] NP, not performed.

TABLE 5. *Effects of porcine competitive-exclusion culture on horizontal transmission of* Salmonella *Cholerasuis between littermates*[a]

Group	No. of rectal samples of *Salmonella* Cholerasuis positive/ no. of rectal samples tested (%)						
	Litter 1b	Litter 2b	Litter 3b	Litter 4b	Litter 5b	Litter 6b	Total
Seeders	26/63 (41) A	28/63 (44) A	NP[b]	NP	40/72 (55) A	NP	94/198 (47) A
Untreated contacts	12/63 (19) B	NP	NP	8/63 (12) A	9/105 (8) B	7/59 (11) A	36/290 (12) A
Treated seeders	NP	NP	20/84 (23) A	20/42 (47) B	NP	9/63 (14) A	49/189 (25) B
Treated contacts	NP	7/84 (8) B	2/84 (2) B	NP	0/84 (0) C	2/63 (3) B	11/315 (3) C

[a] Treated contact pigs were provided an oral dose of 5.0 ml of the porcine competitive-exclusion (PCF1) culture containing 10^9 CFU/ml within 4 h of birth. Untreated contact pigs were neither challenged with *Salmonella* Cholerasuis nor provided PCF1. Seeder pigs were challenged with 10^7 CFU of *Salmonella* Cholerasuis. Piglets remained on sows until day 14 and were then weaned; individual litters were housed in separate pens until the termination of the experiment. Values with different letters in the same column are significantly different ($P < 0.05$).

[b] NP, not performed.

swine production after the administration of the PCF1 culture to pigs as neonates.

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